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# **The use of erythropoiesis stimulating agents (ESAs) is safe and effective in the management of anemia in myelofibrosis patients treated with Ruxolitinib**

Running title: Erythropoiesis-stimulating agents (ESAs) are effective in improving anemia in MF patients treated with Ruxolitinib without significant toxicities

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## Abstract

Erythropoiesis-stimulating agents (ESAs) have been used to treat anemia in myelofibrosis (MF) setting, with response rates around 40-50%. Ruxolitinib is approved for intermediate 2 and high DIPSS risk MF and effectively reduces spleen size and symptoms. However, anemia usually does not improve during Ruxolitinib therapy and even worsen in 40% of patients. We report a retrospective multicenter experience of combined use of ESAs and Ruxolitinib in 59 MF patients. Median age at ESA start was 69 years and 71% and 22% of the patients were at intermediate-2 and high risk according to DIPSS, respectively. Nine patients started ESAs together with Ruxolitinib and 50 soon after. Overall, 52.5% of patients were transfusion-dependent at ESAs start. Anemia response (AR) rate according to IWG-MRT criteria was 52.5% and 95% of patients were still responding at 4 years. Seventy-eight per cent of the patients responded to Ruxolitinib in terms of spleen size, 61% of whom also achieved an AR to ESAs. Only one patient responsive to Ruxolitinib had a spleen increase during ESAs treatment. Patients with endogenous erythropoietin levels ( $<125$  U/L) at ESAs start had a significantly higher AR rate as compared with the others (63% vs 20%,  $p=0.008$ ). No thrombotic events or other toxicities occurred over ESAs treatment. Overall survival was 62% at 4 years and at multivariate analysis was affected by DIPSS and transfusion dependency at ESAs start. In conclusion, ESAs seem effective in improving anemia in MF patients treated with Ruxolitinib, without significant toxicities.

## Key words

Myelofibrosis, anemia, erythropoiesis stimulating agents, ruxolitinib, erythropoietin

## Introduction

Anemia is one of the most common clinical features and represents a negative prognostic factor for survival in patients with myelofibrosis (MF)<sup>1</sup>. In clinical practice erythropoiesis stimulating agents (ESAs) are a possible therapeutic strategy for the management of anemia in this setting; early monocentric retrospective studies, on small patient series, showed an anemia response (AR) rate in 23- 60% of patients<sup>2-6</sup>. A larger retrospective report has been recently published by the Spanish group on 163 patients showing a response rate of 50%, higher in females and in patients with leukocytosis or low serum endogenous erythropoietin (EPO) and/or ferritin levels<sup>7</sup>.

Ruxolitinib is an oral JAK1 and JAK2 inhibitor and currently represents the main approved treatment for MF at intermediate 2 or high International Prognostic Scoring System (IPSS)/Dynamic-IPSS /DIPSS risk. Two prospective randomized trials have demonstrated that Ruxolitinib is highly effective in reducing the spleen size and controlling the symptoms of MF, this resulting in a marked improvement in the patients' quality of life and possibly in a prolonged survival<sup>8-10</sup>. However, consistent with its known mechanism of action, one of Ruxolitinib's main side effects is anemia, which occurs in at least 40% of patients and can be a limiting factor for treatment tolerability and optimal effective dosage, mostly in the first 12-24 weeks of treatment. Moreover, Ruxolitinib has not been reported to improve hemoglobin (Hb) level in patients already anemic at treatment start<sup>8,9</sup>. Despite the opposite effect on JAK2 pathway of Ruxolitinib and ESAs and the discouraged use of the latter in COMFORT I study, some responses to ESAs were seen in a small patient group (13 patients) from COMFORT II study and reported in a post hoc analysis<sup>11</sup>.

Here we present our retrospective multicenter experience on a combination therapy with Ruxolitinib and ESAs on the largest patient series published so far.

## Methods

In our Institutions ESAs (epoetin alpha/beta/zeta or darbepoetin) were administered off-label (after obtaining patient written consent and local pharmacy approval) to treat anemia (Hb <10 g/dL) of MF patients in the absence of other approved treatments in this setting.

Ruxolitinib was given to MF patients at IPSS intermediate 2 or high with splenomegaly according to the approved indications in Italy; few patients at IPSS intermediate 1 received it in a compassionate use program.

We retrospectively evaluated 59 consecutive patients who received Ruxolitinib for symptomatic disease or splenomegaly combined with ESAs for the management of anemia.

Patients could have received ESAs for anemia together with Ruxolitinib or after Ruxolitinib start for new onset or worsening anemia during Ruxolitinib treatment.

Epoetin alpha/zeta, beta and darbepoetin were administered subcutaneous according to conventional schedules: a starting weekly doses of 40000 UI, 30000 UI and 150 mcg, respectively, that could be increased in case of no response up to 80000 UI and 300 mcg, respectively.

The anemia response (AR) rate was defined according to the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria: obtainment of transfusion independence in transfusion dependent patients or sustained Hb increase of at least 2g/dl in the transfusion independent ones.<sup>12</sup> Hematological improvement was defined as transfusion decrease of >50% or sustained Hb increase of 1-2g/dl in transfusion independent patients<sup>2</sup>.

Ruxolitinib was given at the standard dosage with adjustment in case of hematological and extra-hematological toxicity, as provided by manufacturer recommendations.

The study was approved by our local ethic committee.

## **Statistical methods**

Patients' characteristics were compared using Pearson chi-square test for the categorical variables and the Kruskal-Wallis test for the continuous ones. Overall survival (OS) was estimated from ESA start until death or last follow-up by Kaplan Mayer method; any statistical difference between curves was assessed by log rank test. Response duration was defined as the time from the achievement of AR to treatment failure and was estimated by Kaplan Mayer method. Patients who discontinued ESA still in response were censored at the time of treatment discontinuation. Univariate and multivariate analyses were performed using the Cox model. All statistical analyses were conducted using SPSS.

## **Results**

### **Patients characteristics**

We included 59 patients diagnosed with MF: 39% primary MF (PMF), 27% secondary to polycythemia vera (PPV-MF) and 34% to essential thrombocythemia (PET-MF). Thirty-five patients (59%) were male. Mutational status was evaluated in 50 patients: the majority of patients was JAK2 V617F positive (84%) whereas 14% were CALR mutated and only 1 patient harbored MPL mutation. Median time from diagnosis to ESAs start was 19 months (range 0-81) and to Ruxolitinib start 14 months,(0-70).

### **Baseline characteristic at ESAs start (Table 1)**

Median age at ESAs start was 69 years (range 48-81). The distribution of patients according to DIPSS was: 7% intermediate-1, 71% intermediate-2 and 22% high risk. Median Hb at ESAs start was 8.7 g/dL (range 6 – 10 g/dL) and 31 patients (52.5%) were red blood cells (RBC) transfusion-dependent. Median baseline ferritin level was 339 ng/mL (range 5 – 6134 ng/mL).

Nine patients received ESA together with Ruxolitinib and 50 after being on Ruxolitinib for a median time of 4 months (range 1-38 months).

Median baseline endogenous EPO level was 70 U/l (range 2-674 U/L). Thirty per cent of the patients received epoetin alpha, 51% epoetin beta, 10% darbepoetin and 9% epoetin zeta. Patients received ESAs for a median time of 15 months (range 2-55).

Forty-five patients (76%) were already significantly anemic (Hb <10 g/dL) before starting Ruxolitinib. Nine of them received ESAs together with Ruxolitinib and 36 after a median of 5 months (range 1-38). Out of these 45 patients 15 were already RBC transfusion dependent at Ruxolitinib start whereas 17 became transfusion dependent over treatment (one patient started ESAs 10 months after ruxolitinib being still anemic but not anymore transfusion dependent).

The remaining 14 patients became anemic after receiving Ruxolitinib and were subsequently started on ESAs after a median time on Ruxolitinib of 2 months (range 1-20).

### **Response to ESA**

Median follow up from ESA start was 48 months (range 11-68). AR rate was 54% (32/59 patients). Median dose at AR for epoetin alpha/beta/zeta was 40000 U/week, for epoetin beta 30000/week and for darbepoetin 150 mcg/week. In 6 patients ESAs dose was increased due to lack of response or response loss: 4 of them subsequently achieved an AR, 1 achieved an Hb increase without meeting AR criteria and 1 did not respond. Median time to AR was 4 months (range 1-13 months) and 95% of the patients were still responding at 4 years (Figure 1). Among the 32 responsive patients, 24 were still on ESAs at the last follow-up, including 7 patients who died still in response, whereas 8 patients discontinued ESAs for response loss (4 cases) or sustained anemia response (4 cases)

In order to clarify the Ruxolitinib impact on AR to ESAs we divided patients according to the timing of ESAs start. Twenty-nine patients started ESAs after being on Ruxolitinib for more than 3 months (median time for ruxolitinib induced anemia resolution), whereas 30 started ESAs together with Ruxolitinib or within the first 3 months. AR rate was 55% in the first group and 53% in the second one, respectively.

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We also analysed separately the 45 patients already anemic before ruxolitinib start who received ESAs after or together with Ruxolitinib, and we observe an AR rate of 51% (23/45).

AR rate seemed somewhat higher in the 46 patients (78%) responding to Ruxolitinib in terms of spleen size: 61% vs 33% in non-responding patients, even if *p* value was not statistically significant (*p* 0.088).

Lower EPO endogenous levels at baseline were a significant predictor of AR, with 63% of patients with EPO <125 UI/dl responding vs 20% in the EPO >125 UI/dl group (*p*= 0.008). We were unable to identify any other predicting factors of response to ESA. In particular DIPSS, serum ferritin level at baseline, blood counts, mutational status for JAK2, CALR or MPL, age, disease duration and transfusion dependency did not have any significant impact on response. Indeed 61% of transfusion dependent patients responded, as compared to 46% of the transfusion independent ones (*p* 0.253).

Of note, a further 15% of patients achieved an Hb improvement without meeting the criteria of AR, leading to an overall response rate of 69% (41/59 patients).

#### Toxicity

No thrombotic events and hematological or extra-hematological toxicity except for mild nausea was reported over treatment with ESAs. A spleen increase during ESAs treatment in patients responding to ruxolitinib was observed in 1 case only.

#### Overall survival

Twenty-one patients (35,5%) died, 7 after leukemic evolution, 5 for infection, 2 for acute myocardical infarction (not being on ESAs treatment), 1 for end stage renal failure, 2 for second cancer colon cancer, 1 for gastrointestinal bleeding, 1 for trauma, 1 after undergoing allogeneic stem cells transplantation and 1 for unknown cause. Survival rate at 2 and 4 years from ESAs start was 78% and 62% (Figure 2), respectively, and at multivariate analysis it was significantly affected by DIPSS, and transfusion dependency whereas the impact of AR was border line (Table 2).

### **Discussion**

Anemia is one of the main issues in MF and there is an unmet need for approved drugs acting in this setting. Moreover anemia can be a limiting factor for effective tolerated dosage and patient compliance to Ruxolitinib, the only approved JAK1/JAK2 oral inhibitor for treatment in MF.

Here we report a multicenter retrospective experience on the use of ESAs for the management of anemia in MF patients receiving Ruxolitinib. ESAs are a valid option for anemia in MF patients and have been routinely employed in our centers in approximately 100 MF patients when Ruxolitinib was not available yet or contraindicated (unpublished data). The AR rate obtained (49%) was quite similar to that recently reported in a larger series by the Spanish group<sup>7</sup>. More recently we have extended ESAs to anemic MF patients receiving Ruxolitinib. Since Ruxolitinib prescription in Italy until now is limited to patients with splenomegaly and Intermediate 2 / High IPSS score and our patients were also anemic, the majority of them had a rather poor prognosis. Nevertheless, 54% of the patients achieved an AR, similarly to data reported by others with ESAs<sup>2-6</sup> alone in reports including more patients with less unfavorable prognostic features. Importantly 59% of our responsive patients were previously transfusion-dependent and could avoid transfusions with ESAs treatment.

ESAs use was discouraged in the first Ruxolitinib clinical trials<sup>11</sup>, for a concern on its possible activation of the JAK pathway, potentially resulting in increased spleen size and thereby confounding the efficacy analyses of spleen response. For the same reason, ESAs might have been expected to be less effective in the presence of JAK 2 inhibition. However, JAK 2 is probably not completely inhibited by Ruxolitinib at the therapeutic concentrations and the reduction of splenomegaly and of inflammatory cytokines induced by Ruxolitinib could synergize to ESA in improving anemia. Indeed the AR rate was higher in patients responding to Ruxolitinib than in patients not responding. Moreover, while previous studies reported a median erythroid response duration of about 20 months<sup>7</sup>, in our MF patients AR seemed to be long-lasting, with median response duration unreached and 90% of patients still in response at the median follow up of 48 months. Finally, we observed a comparable AR in transfusion dependent and non-dependent patients, differently from what previously described with ESAs alone.<sup>7</sup>

In COMFORT studies Ruxolitinib induced anemia resolved in the majority of patients within 12 weeks<sup>13</sup> but no anemia improvement was reported in the patients already anemic or transfusion dependent pre-ruxolitinib start. In our series the majority of patients were already anemic before Ruxolitinib start and received ESAs after a median of 2 months after commencing Ruxolitinib, achieving a response after a median of 3 further months of ESAs treatment. Besides, a comparable (~50%) AR rate was observed in the group of patients starting ESAs more than 3 months after Ruxolitinib (when ruxolitinib induced anemia was likely resolved in most cases). Therefore, these data suggest that AR in the majority of cases was correlated with ESAs treatment and not only to the spontaneous resolution of the early ruxolitinib toxicity on red cells progenitors.



Our data also confirm the endogenous EPO level as a good predictor of response, which can easily be used to select patients to be challenged with ESAs treatment.

Importantly, besides the high AR rate, we did not see any negative impact of ESAs on response to Ruxolitinib. Indeed 78% of our patients experienced a spleen reduction with ruxolitinib, as expected from literature data<sup>8,9</sup> and one responding patient only had an increase in spleen size during ESAs treatment. Moreover, no thrombotic event during ESAs treatment was observed.

Overall survival was at least comparable to the one reported in COMFORT studies<sup>13,14</sup>. In particular, a response to ESAs was associated with a trend towards better survival, together with DIPSS and transfusion dependency, as shown by others<sup>7</sup>.

Our favorable results in terms of AR are in line with previous reports on ESAs alone; besides, a further 15% of patients obtained an “anemia improvement”. Moreover, the longer response duration and the higher response rate in transfusion-dependent patients, compared to results with ESAs alone, could suggest a synergistic more than antagonistic activity of ESAs and Ruxolitinib, possibly due to the activity of Ruxolitinib in reducing splenomegaly and inflammatory symptoms. Similar results have been recently reported by the French group on a smaller patient series, in abstract form only<sup>15</sup>.

In conclusion, ESAs seem effective in improving anemia in MF patients treated with Ruxolitinib, without significant toxicities. The predictive value of endogenous EPO levels could help to identify which MF patients are more likely to benefit from ESAs treatment. A larger prospective trial is needed in order to include ESAs in the treatment options for the management of anemia in this setting of MF patients.

### **Authorship Contributions**

E.C. designed research, followed the patients, analyzed data and wrote the paper; D.F. designed research and wrote the paper; D.C., E.M.E., V.M., G.A.P., N.P., E.B., C.F., M.C., G.L., M.M., M.M. followed the patients and collected data. M.B. supervised the research and provided funding. All authors reviewed and approved the manuscript.

## Conflict of Interest Disclosures

No conflict of interest to disclose

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## Tables

**Table I – Patients characteristics at the start of ESA therapy**

	n (%) or n (range)
Age, years	69 (48-81)
Gender (males/total)	35/59 (59.3%)
PMF	23/59 (39%)
PPV - MF	16/59 (27.1%)
PET - MF	20/59 (33.9%)
JAK2 V617F	42/50 (84%)
CALR	7/50 (14%)
MPL	1/50 (2%)
Transfusion dependency	31/59 (52.5%)
Haemoglobin (g/dl)	8.7 (6.0 – 10)
Leukocytes count (x 10 <sup>9</sup> /L)	9 (1 – 31)
Serum ferritin levels (ng/mL)	344 (5 – 6134)
EPO serum levels < 125 UI/mL	27/42 (64.2%)
DIPSS Int - 1	4/59 (6.8%)
DIPSS Int -2	42/59 (71.2%)
DIPSS High	13/59 (22%)
Darboepoetin	5/59 (8.4%)
Erythropoietin	54/59 (91.6%)

**Table II**

<b>Multivariate analysis of patient overall survival</b>			
	<b>HR</b>	<b>IC 95%</b>	<b>p value</b>
Anemia response yes vs no	0.43	0.17-1.19	0.076
DIPSS high vs intermediate 1/2	3.68	1.17-1.09	0.017
RBC transfusion dependency	4.00	1.41-11.29	0.006

Figure Legends

Figure 1. Anemia response duration, median not reached.

Figure 2. Overall survival, median 61 months.

Figures

Figure 1

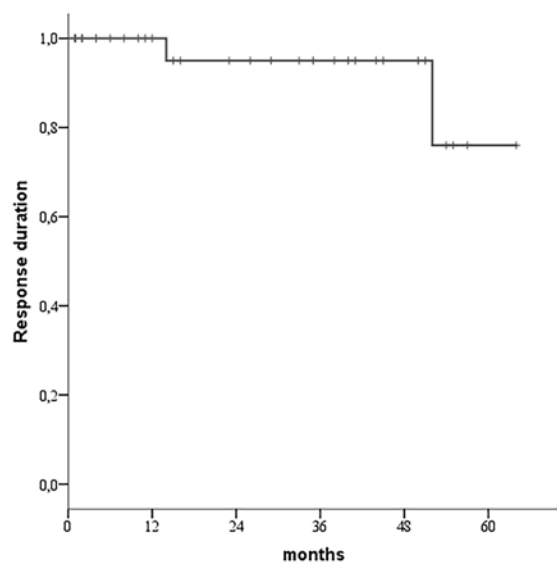


Figure 2

